Increased Somatostatin-axon density in Layer 1 does not predict input density

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Introduction

Why study Layer 1 and Somatostatin-cells (SST)?

- Layer 1 (L1) is a hub of activity. Lots of exchange of information occurs here[1]
- SST-expressing inhibitory neurons synapse onto and control the activity of the main excitatory neurons of the cortex (pyramidal neurons)[1]
- SST neurons have high axon density in L1, suggesting they synapse onto the apical dendritic tuft of pyramidal neurons[2]
- SST axons in L1 disappear during learning, suggesting their connections are plastic
- Our method of analysis would be a way to quantitatively determine changes during learning

Questions:

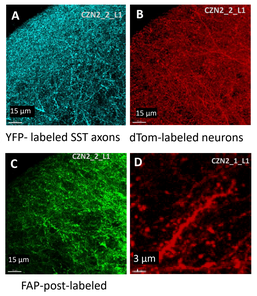
- Which part of the pyramidal neurons do SST-cells preferentially innervate? The dendritic shaft or the dendritic spines?
- Does the location of the dendrite influence the density of SST input?

Methods

We used the image analysis software, Imaris, to reconstruct presynaptic SST neurites and the synapses (puncta) and *spiny* dendrites associated with a pyramidal neuron, from a fluorescence image. We then collected the points of contact between the puncta and SST at different distances away from the dendritic shaft (0.1, 0.5, 1.0, 2.0, and 3.0 µm). The dendrites were then labeled with their location in the cortex (upper-L1, lower-L1, and L2).

Labeling of presynaptic-SST Neurons

Ai3 mice contain the gene for enhanced yellow fluorescent protein (EYFP) but cannot express it without Cre recombinase (Cre)
SST-IRES-Cre mice have Cre present in only SST-expressing cells
Ai3 x SST-Cre SST-cells will express YFP

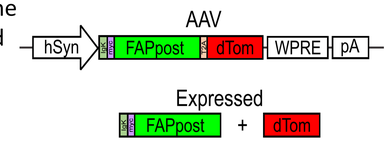


YFP- labeled SST axons dTom-labeled neurons

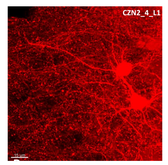
FAP-post-labeled synapses

Labeling of Synapses

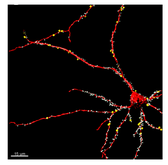
Cre-independent FAPpost and dTom expression → Cells infected by the AAV virus will express FAPpost and dTom. FAPpost will localize to synapses and fluoresce.



Methods (*cont.*)

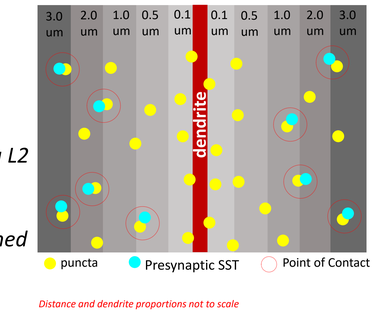


dTomato fill of neurons infected by AAV



Reconstruction of a L2 Pyramidal neuron with spots representing assigned and unassigned puncta

Distance Threshold Test



Distance and dendrite proportions not to scale

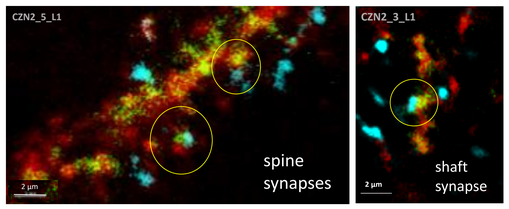
Puncta with a point of contact were SST-assigned.

SST-puncta density = $\frac{\text{\# points of contact}}{\text{dendrite length}}$

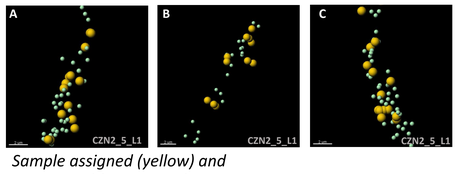
How does SST-assigned puncta density change as the distance from the dendritic shaft increases?

Results

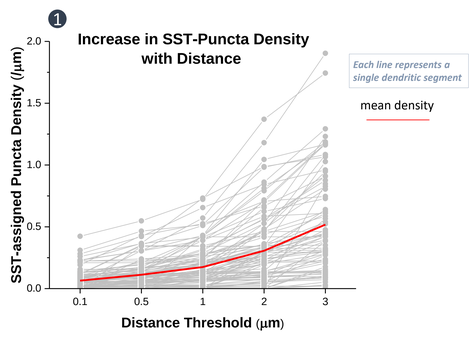
SST-input density increases as the distance from the shaft increases



The overlap between the green and blue fluorescence is a point of contact

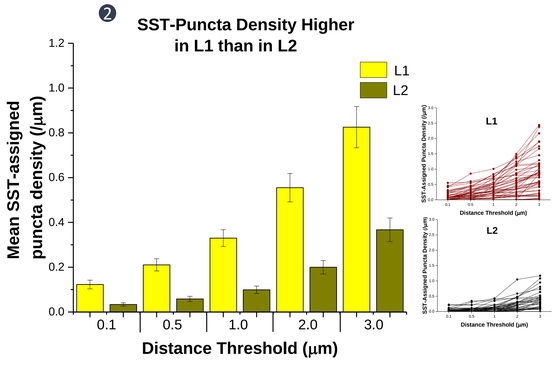


Sample assigned (yellow) and unassigned (mint green) spots

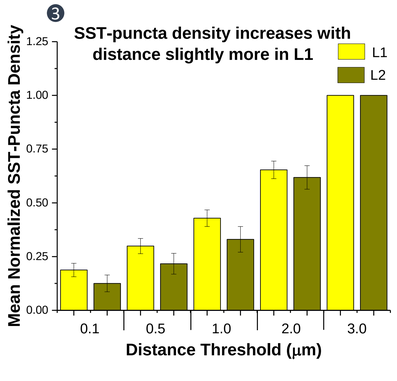


As the distance threshold is increased, the SST-assigned puncta density also increases, suggesting that SST-input density is greatest furthest away from the dendritic shaft (on spines).

Overall SST-input density is higher in layer 1 than it is in layer 2



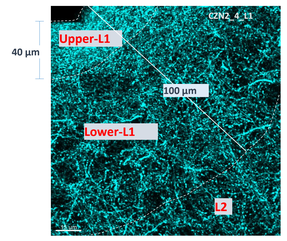
SST-input density is higher in Layer 1 than in Layer 2 at **all distance thresholds.**

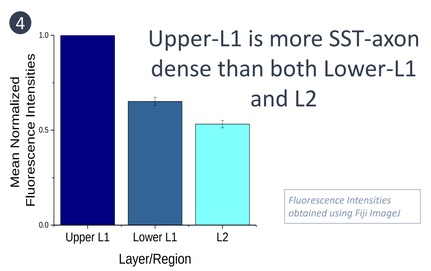


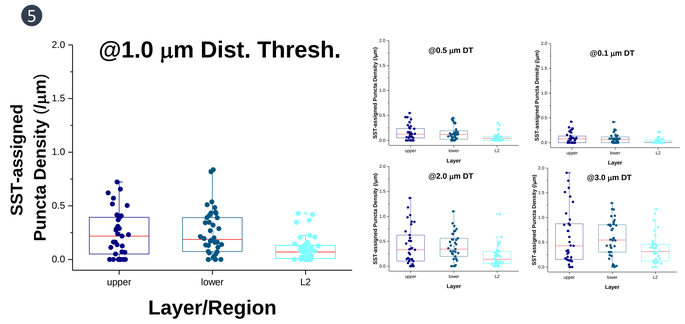
The change in SST-puncta density with distance is slightly more in L1.

Results

SST-axon density does not predict input density onto the apical tuft of pyramidal neurons







Similar SST-input densities in upper and lower L1 despite SST-axon density differences

Conclusions

- SST inputs synapse onto both shafts and spines
- SST axons are most dense in upper L1
- SST input density on pyramidal dendrites is similar across L1
- SST input density is lower in L2
- SST axon density in upper L1 may be targeting other cell types
- Digital input assignment has a low false positive rate because SST axon density is uncorrelated with input assignment

References & Acknowledgements

1. M. E. (2013). The yin and yang of cortical layer 1. Nat. Neurosci. 16, 114–115. 1Larkum0.1038/nn.3317
2. Wang Y, Toledo-Rodriguez M, Gupta A, Wu C, Silberberg G, et al. (2004) Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. J Physio 561: 65–90. 10.1113/jphysiol.2004.073353
3. Urban-Ciecko J., Barth A.L. Somatostatin-expressing neurons in cortical networks. Nat. Rev. Neurosci 2016;17:401–409.

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